PARTICLE CONCENTRATION METHOD

DESCRIPTION

TECHNICAL DOMAIN AND PRIOR ART

This invention relates to the domain of treatment and particularly concentration of small particles. These particles may be biological particles such as liposomes, animal or vegetable cells, viruses or micro-organisms, macromolecules, for example such as DNA, RNA or proteins, or inorganic particles such as microballs. Application domains may then be chemical or biomedical analysis or quality control (calibration of micro-particles).

Known approaches in terms of particle cell sorting, such as flow cytometry, reach their limits particularly for the analysis of rare or very minority cell populations.

The technique of optical clamps is based on the confinement of a particle (microball, or cell or macromolecule) by the intensity gradient generated at the waist of a continuous laser beam. For example, it is described in the article by "Ashkin and Dziedic" entitled "Observation of radiation-pressure trapping of particles by alternating light beams" published in Physics Review Letters, 54(12), 1985. This operation is made possible by balancing of radiation pressures. Once this operation has been done, the particle is displaced by displacing the beam.

Thus, displacement distances on this type of device are usually limited to a few hundred microns.

Finally, treatment and particularly concentration of particles is not possible.

Figure 1 shows the principle of such a device.

5   A particle 2 is confined by a beam 4 in a liquid medium 6.

Figure 2 is a diagram showing a force field generated by the device, on each side of the laser beam 4; the particle is confined in a mechanical force field
10   (induced by the radiation pressure provoked by the electrical field of the laser) which makes it possible to trap it.

This type of device has a disadvantage: displacement of particles is based on use of a
15   dedicated mechanical system, which may be difficult and expensive to set up.

Recent work, for example such as that described in the article by T. Tanaka et al, published in Applied Physics Letters, Vol. 77, p. 3131, 2000,
20   makes use of guided optical devices, and suggests the possibility of designing a device for displacement of cells by optical forces; this technique is limited to objects very much smaller than a biological cell (balls and colloids with a size of the order of a few
25   microns).

As illustrated in Figure 3, this device uses a waveguide 10 with a strip made on a substrate 12. A particle is displaced by a force with photonic pressure, which is proportional to the light intensity
30   at the particle. The particle is held in place in the

guide by a force that is proportional to the gradient of the intensity.

If the waveguide is single mode, there is a maximum light intensity at the location at which the particle will be trapped.

Furthermore, if it is desired to concentrate objects such as biological cells, special care must be taken not to damage them. Concentration methods involving liquid flows (for example concentration by retention on a membrane) cause the problem of a possible overpressure that is highly damaging to cells and that can occur following a blocking in the guide circuit.

The use of optical clamps, although less damaging for cells, is also not feasible since each clamp can only handle a single object and in this case it will be necessary to make one manipulation for every particle to be displaced. This method is fairly tedious and requires qualified personnel and / or very delicate computer control.

The problem arises of finding a method and a device for concentrating particles simply and efficiently.

PRESENTATION OF THE INVENTION

The invention relates to systems for concentrating particles or objects, for example with biological interest.

The invention uses at least one waveguide, for example with a length between a few micrometers and a few centimetres.

Particles may then be concentrated in one or several clusters: the particles to be concentrated are firstly placed close to a portion of the guide. Light radiation is then injected into the waveguide

5 which produces forces that attract all particles and concentrate these particles in a cluster.

In particular, these forces may be short range optical forces (of the order of a few micrometers and / or forces related to convection of a fluid above

10 the guide, which have a longer range (several tens of micrometers).

The invention may also use several waveguides so that particles can be concentrated in one or several clusters. These clusters may be distributed

15 on one or several guides, or possibly on each of the waveguides. According to one variant, this can be achieved by adding light radiation into each of the waveguides, during the step in which light radiation is inserted. According to another variant, light radiation

20 can be inserted into one or several particular guides among the set of waveguides.

The said waveguides may be put adjacent on a support without intersecting. They may also be multiplexed and joined together at least at one

25 concentration point. In this case, the injection step can be used to concentrate the particles into a single cluster on this concentration point.

Once concentrated on one or several waveguides, the particles may tend to displace; the

30 particle cluster(s) formed may then tend to dislocate.

According to one characteristic of the invention, stationary waves may be used to prevent this displacement. These stationary waves may be formed from the light radiation used, for example using a

5   diffraction grating or an optical loop formed by the waveguide.

The use of stationary waves may also help to hold particles concentrated in several clusters fixed on the same waveguide.

10   The said injected light radiation may be between the ultraviolet and infrared. It is chosen particularly according to criteria such as the nature of the particles to be concentrated, and the velocity at which this concentration has to be done.

15   The particles to be concentrated may be objects derived from a micro-manufacturing method for example such as microballs with a size between a few nanometres and about a hundred micrometers. These particles may also for example be biological elements

20   such as cells, macromolecules such a proteins, DNA, RNA.

According to one particular characteristic of the method according to the invention, the particles to be concentrated may be marked before being placed on

25   the said support used. This provides means for increasing the difference in the index of particles with respect to their environment and can thus improve their concentration when light radiation is injected.

For example, when the particles to be

30   concentrated are cells, marking may be done by other marking particles, for example based on metal such as

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gold or polymer. These marking particles may for example be microballs.

The method according to the invention may be done in a liquid medium such as water or a buffer
5   solution, or a cell suspension medium.

The method may be carried out in such a medium particularly when the particles to be sorted are biological elements.

The invention also relates to a particle
10   concentration device including one or several waveguides, the waveguides being surrounded on each side by at least two diffraction gratings.

BRIEF DESCRIPTION OF THE FIGURES
15   This invention will be better understood after reading the description of example embodiments given purely for information purposes and in no way limitative, with reference to the appended figures wherein:

20   - Figures 1, 2 and 3 illustrate techniques according to prior art,
     - Figures 4A and 4B illustrate a general example of a method for concentration of particles according to the invention,
25   - Figures 5A and 5B illustrate examples of devices for concentrating particles into one or several stationary clusters on the same waveguide,
     - Figures 6A and 6B illustrate an example of the method for concentration of particles according
30   to the invention in which several adjacent waveguides are used,

- Figure 7 illustrates an example of a device for grouping several clusters of particles distributed on different waveguides, into a single particle cluster,

5   - Figure 8 illustrates an example device used to facilitate concentration of particles on a waveguide,

- Figure 9 illustrates an example device used to observe the concentration of particles on a

10   waveguide,

- Figures 10A to 10D, 11 illustrate an example of a method for making waveguides that can be used during the method according to the invention,

- Figures 12A to 12D, 13, 14A to 14C show

15   experimental results obtained using the method and a device according to the invention.

Identical, similar or equivalent parts of the different figures are marked with the same numeric references so as to facilitate comparison between one

20   figure and the others.

The different parts shown in the figures are not necessarily at the same scale, to make the figures more easily readable.

25   **DETAILED PRESENTATION OF PARTICULAR EMBODIMENTS**

A general example of the method according to this invention will now be described with reference to Figures 4A and 4B.

This method is used to concentrate or to

30   group a set of particles 100. Grouping may be done so as to form one or several particle clusters.

Particles mean organic or inorganic elements or objects with a size varying from 5 nanometres to 100 micrometers. These particles may for example be biological elements such as liposomes,

5   viruses, micro-organisms, animal or vegetable cells, macromolecules for example such as proteins, DNA, RNA or micro-objects, for example such as microballs based on a metal or a dielectric material.

The concentration method according to the

10   invention is done on a support 104, for example made of glass or silicon, with an optical index preferably different from or very different from the optical index of the particles that are to be grouped. This support 104 comprises at least one integrated multi-mode or

15   single mode waveguide 108 for example with a length of between a few micrometers and a few centimetres.

According to a first step in the method, the particles 100 are placed firstly in an area 102 close to and / or on the waveguide 108, using a manual

20   or automated method.

Then, using an optical device 112 that may or may not be integrated into the support 104, light radiation R is injected into the optical guide. The injected radiation has a wavelength between the near

25   ultraviolet and infrared, for example between 300 nm and 1200 nm (Figure 4A).

The light radiation R may be chosen particularly depending on the type of particles to be concentrated, and possibly the velocity at which these

30   particles are to be grouped.

For example, in the case in which the particles are biological elements such as cells, a wavelength between the red and infrared could be used, for example using a YAG laser with a wavelength of
5   1064 nm. The radiation power could be of the order of a few tens of milliwatts to a few hundred milliwatts, for example between 50 mW and 1 W, for example close to 150 mW.

The light radiation R through the waveguide
10 108 creates forces $\vec{f}$ towards the waveguide. These forces may attract and concentrate particles 100 on the waveguide 108. These particles 100 then form a cluster 106 (Figure 4B).

The velocity at which the particles are
15 grouped can vary depending on the mass, the volume, optical index of the particles, and the wavelength of the light radiation used.

If the light radiation injection step is prolonged, the cluster 106 may tend to be dislocated.
20 Particles can tend to displace along the waveguide 108 along the direction of propagation of light radiation.

If it is required to prevent this phenomenon, according to one variant, the injection of light radiation can be stopped as soon as the cluster
25 106 of particles 100 is formed.

According to another variant, the particles 100 can be blocked once they are on the waveguide, for example using stationary waves. These stationary waves may be formed by transforming the light radiation R in
30 the waveguide, for example using one or several

diffraction gratings, or by making an optical loop system.

Figure 5A relates to a device like the one illustrated in Figures 4A and 4B. The waveguide 108 5 also comprises an integrated diffraction grating 200 used to transform light radiation produced by the optical device 112 into stationary waves.

The use of stationary waves through waveguide 108 can also concentrate and maintain 10 particles in several clusters 202, 204, 206 formed along the same guide 108.

These clusters 202, 204, 206 are located at locations on the waveguide at which the light intensity is maximum.

15 The curve C illustrated in Figure 5A represents the variation in the amplitude of a stationary wave derived from the radiation R, along a z axis transverse to the waveguide and along the direction of propagation of the radiation R. The 20 maximum values of the curve C correspond to the locations on the waveguide 108 at which particle clusters are grouped.

According to one variant, a stationary wave can be made in the waveguide using a guide forming at 25 least one loop 210 like that illustrated in Figure 5B. This type of loop can enable light radiation, when it passes along the waveguide, to adopt one or two different trajectories. Thus, two light waves with the same length and the same amplitude propagating in 30 different directions, can come into contact and interfere.

The method for concentration of particles according to the invention may also be used with a device comprising several waveguides. It is required to concentrate a group of particles placed in an area close to the different waveguides into one or several clusters using such a device.

For example, this variant may be produced using a support comprising several adjacent waveguides with certain spacing between them. These waveguides may have different lengths and / or different widths and may be made of different materials.

A first method of grouping particles consists of injecting light radiation into one of the waveguides. If the distance between the different adjacent waveguides is small enough, coupling between the guides can occur and the light radiation then propagates in one or more of the other guides. When coupling occurs, the particles may be attracted by forces from the different guides and may be concentrated in different clusters distributed on these different guides.

However, when coupling occurs between several guides, it is possible that a particle oscillation phenomenon can occur from one waveguide to another. Another phenomenon can also occur in which particles remain blocked between two waveguides.

In order to optimise the concentration method and prevent these two phenomena, the distance between the waveguides may be fixed equal to a value greater than a minimum distance that will enable coupling between the different guides. However, this

minimum distance depends particularly on the geometric properties of the guide, the refraction index of the materials from which it is made, and the wavelength of the radiation used. It may be of the order of a few micrometers, for example more than 5 µm, to several tens of micrometers, for example between 5 µm and 50 µm.

An example of a device comprising several adjacent waveguides 211, 212, 213 at a spacing of a distance e, is illustrated in Figure 6A. The distance e is fixed so as to avoid coupling between the different waveguides, at a value for example of the order of one or several tens of micrometers, for example 10 or 20 µm. Different light radiation R_1, R_2, R_3 can be injected into each of the waveguides 211, 212, 213, to concentrate the particles 100 located close to the waveguides 211, 212, 213 (Figure 6A). This provides a means of concentrating the particles 100 into different clusters 214, 215, 216 distributed on the different waveguides (Figure 6B).

At the end of this method, it will be possible to group the different particle clusters distributed on several waveguides into a single cluster.

To achieve this, a support 104 can be used including several adjacent and multiplexed waveguides. These guides join together at a concentration point 220 (Figure 7). The injection of light radiation through each of the waveguides causes concentration of particles into several clusters distributed on the different waveguides. Then, if the emission of light

radiation is prolonged, the particles 100 tend to displace along radiation propagation directions (indicated by the arrows in Figure 4). The particles 100 then move towards the concentration point. The

5   particles 100 are concentrated in a single cluster.

One method of improving the concentration of particles consists of producing additional radiation forces $\vec{F}_{add}$ on the particles 100, added to forces created by the waveguide. These additional radiation

10   forces $\vec{F}_{add}$ may for example be produced by diffraction gratings 232 located on each side of the waveguide through which additional light radiation R' is injected (Figure 8).

The method according to this invention may

15   be applicable to biological particles such as animal or vegetable cells to be concentrated. A support, for example made of glass, comprising at least one waveguide will be used to concentrate biological particles.

20   The support may be immersed in a liquid solution, preferably biocompatible, to preserve cells.

In a heterogeneous cell sample, an attempt is made to isolate a given sub-population characterised by a specific phenotype, for example the presence of a

25   certain type of surface macromolecules, for example such as proteins. Furthermore, probe molecules such as antibodies are available capable of recognising and bonding with these phenotypic markers with a very strong affinity. In the case of antibody type probe

30   molecules, the phenotypic markers are called antigens. Antibodies are fixed by means known to those skilled in

the art to balls chosen for their particular characteristics, for example gold balls. These functionalised gold balls are then grafted onto the surface of cells, for example these cells may be
5   lymphocytes isolated from blood and that are to be concentrated.

A group of marked cells is sampled firstly, for example, using a pipette. The next step is to place the said sample in a support receptacle. This
10   receptacle may be a chamber, for example such a Gene Frame ® type chamber. This self-sticking chamber is very simple and has a joint system impermeable to gas, providing resistance at high temperatures. The receptacle is not limited to this type of chamber.

15   The cells group may be transferred from the receptacle to a zone placed close to the waveguide, for example one or several capillaries.

The next step is to inject light radiation through said waveguide. The radiation used during a
20   cell sort would preferably be inoffensive towards the cells. Thus, the light radiation used may be laser radiation emitting at a wavelength between far red and near infrared, for example between 1000 nm and 1200 nm, for example close to 1064 nm.

25   The passage of laser radiation through the waveguide creates forces directed towards the guide to attract the cells. The cells are then concentrated into a cluster.

In general, observation means may be
30   provided, for example a CCD camera located above the

guide 108.   These means enable monitoring of the sort
made as described above.

Figure 9 shows a particle concentration
system 100 on a support 104 incorporating a guide
5   system according to the invention.   An objective 300
focuses a laser beam R (for example a YAG beam at
1064 nm) in a guide 108.   The particles to be sorted
are contained in a chamber 310 located on a slide 320.
A camera 330 is used to make an image of the
10   concentration, for example using a focusing device or a
zoom 340.   Means 350, 360 (objective, camera) of
forming an image of the transmitted radiation may also
be placed at the output from the device.

A device for implementing a concentration
15   method according to the invention like those described
above, particularly including for example a support and
one or several waveguides, may be integrated in a MEMS
(micro-electromechanical system) or in a lab on a chip.

Waveguides used during the method according
20   to the invention can for example be made by thin layer
manufacturing techniques, or for example by an ion
exchange method.

The support on which these guides are
formed may for example be based on glass.

25   To form the waveguide, the first step is to
cover the support 140 with a metallic thin layer 142,
for example based on aluminium, by a method such as
evaporation or sprinkling.   This aluminium layer is
then covered by a resin layer 144, for example a
30   photoresist resin layer.

To make the patterns of waveguides in the support, the first step is to expose the photoresist resin layer for example using a beam of ultraviolet rays 148 through a mask 146, for example made of
5   chromium.   The mask 146 includes a copy of the waveguide patterns or negatives of the waveguide patterns (Figure 10A).   The areas of the resin layer 144 that are not concealed by the mask have their chemical structures modified.

10   The next step after the exposure step is to develop the layer of photoresist resin 144. Thus, the areas on which the chemical structure was modified by insolation are etched (Figure 10B).

The next step is to etch the aluminium
15   layer through the resin layer (Figure 10C), for example by dipping the support in an aluminium etching solution (AluEtch).   This solution does not etch the resin. Thus, only the previously developed parts are etched.

The resin layer 144 is then removed, for
20   example using acetone (Figure 10D).

An ion exchange step is then carried out to form the waveguides. The support is then immersed in a salt bath containing silver nitrate and sodium nitrate. The proportion between these salts determines the
25   silver content that is exchanged in the glass 140.   The bath generally contains between 10% and 50% of silver depending on the application.   Since the salt melting temperature is about 310°C, the exchange step is carried out at between 320°C and 350°C (Figure 11).

30   The aluminium layer is then removed by etching.   Annealing can possibly be done; the glass

plate is heated without any contact with a bath. This
step enables silver ions to penetrate more deeply
towards the inside of the support.

     A guide 104 like that illustrated in Figure
5 1A can be formed in this way.

     Other methods can also be used, for example
to make a guide on a silicon substrate.

     Braking forces on particles caused by
friction with the upper surface of the guide can be
10 reduced, by coating the guide with a special coating,
for example a thin Teflon based layer.

     Another example embodiment will be given.
In this example, the waveguides used are surface guides
made by a potassium ion exchange (glass slide
15 substrate). These ions are produced at a temperature
of 280°C for an exchange time of 2 h 15. Losses of
these guides are of the order of 0.2 to 0.5 dB/cm at a
wavelength of 1064 nm.

     The displaced particles to be concentrated
20 are glass balls with a refraction index of 1.55 and a
diameter of 2 µm, or gold balls with a diameter of
1 µm.

     The device used is of the type shown in
Figure 9. Light is coupled through the edge using a
25 continuous YAG laser at 1064 nm (P = 10 W) and balls
are observed through the top using a zoom system 340
coupled to a video camera 330 for monitoring their
displacement.

     Experiments carried out on 1 µm diameter
30 gold balls have demonstrated spontaneous grouping of
balls on the guide followed by their displacement at

velocities of the order of 4µm/s along the guide. Similarly, the possibility of grouping and displacing glass balls is demonstrated. Thus, Figures 12A to 14C illustrate:

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- Figures 12A to 12D; displacement of metallic particles over a distance of 70 µm, at t = 0 s, 2 s, 3 s.
- Figure 13: a metallic particles concentration effect.

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- Figures 14A to 14C: progressive grouping of glass balls 101 along a 70 µm portion of the guide, at t = 0 s, 4 s, 8 s successively.